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Genetic Control of Infection of Primary Macrophages with T-Cell-Tropic Strains of HIV-1

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Human immunodeficiency virus type 1 (HIV-1) NDK, a Zairian subtype D virus highly cytopathic for CD4-positive lymphocytes, and the prototype subtype B virus HIV-1 LAV are about 10^4 and 10^5 times more infectious, respectively, for T lymphocytes than for blood-derived macrophages (BDM). Recombinant viruses derived from HIV-1 LAV and HIV-1 NDK were used to determine the genetic control and the step of the virus/cell cycle responsible for infection of BDM with T-cell-tropic viruses. We found that recombinants bearing the envelope glycoprotein of HIV-1 NDK are able to enter more efficiently into BDM than recombinants with HIV-1 LAV envelope glycoprotein. We also found that a genetic region outside of the *env* gene is responsible for production of HIV-1 NDK infectious progeny from BDM. This region consists of the *vif* gene and the C- and N-terminal portions of *pol* and *vpr* genes, respectively. Our results suggest that productive infection of primary macrophages with T-cell-tropic strains of HIV-1 is determined by two different genetic mechanisms: one effective at the virus/cell entry, controlled by the *env* gene, and the second after entry, controlled by genes *vif* and *vpr*. In comparison with HIV-1 LAV, HIV-1 NDK has been able to more easily overcome both restriction mechanisms.

Numerous genetic studies have demonstrated the importance of the *env* gene for HIV-1 host-range tropism (*1–6*) and identified virus entry into the cell as a critical step for infection of both major target cell systems, T lymphocytes and monocytes/macrophages. Our group (*7*) and others (*8–10*) have shown that T-cell-tropic viruses are able to enter and to retrotranscribe their genetic material in blood-derived macrophages (BDM). We concluded that restriction of T-cell-tropic HIV-1 isolates in BDM is controlled at some postentry step of the virus replication cycle. The restriction event has been associated with a step prior to or simultaneous with the integration of the HIV-1 genome (*10*). Recent mutational studies have shown that the matrix (MA) protein $p18^{gag}$ (*11*) and the "nonessential" HIV-1 accessory genes *vif* (*12–14*), *vpr, vpu,* and *nef* (*14–16*) are required for replication of HIV-1 in BDM. Functional characterization of mutant viruses revealed that *MA* and *vpr* genes are necessary for transport and penetration of the HIV-1 nucleoprotein complex into the nondividing nucleus (*11, 17, 18*), and that genes *vif* and *vpu* are involved in maturation of virus particles in primary cells. *vif* is also crucial for synthesis of HIV-1 provirus in infected cells (*12*). These studies have suggested that accessory genes could play a role in control of postentry restriction in BDM.

In the present report we studied the importance of HIV-1 restriction at virus entry into BDM as opposed to the restriction after entry. We focused our interest on the role of the envelope glycoprotein gene and of accessory genes in the control of these processes. To characterize the genetic control of productive infection of BDM with T-cell-tropic strains of HIV-1, we used recombinants derived from HIV-1 LAV, the subtype B prototype virus, and HIV-1 NDK, a Zairian subtype D virus highly cytopathic for CD4-positive lymphocytes (*19, 20*). HIV-1 NDK has a broader host-cell range than HIV-1 LAV and, after entering the cell, it is able to more easily overcome the intracellular block of viral reproduction (*7, 21–24*).

Infection of BDM with HIV-1 NDK was at least 10 times more effective than with HIV-1 LAV (Table 1). At least 10 ng of $p24^{gag}$ of HIV-1 NDK was necessary to productively infect a culture of 5×10^5 BDM. This threshold value was 10,000 times higher than the minimal infectious dose for peripheral blood lymphocytes (PBL) or MT4 lymphocytes. Quantity of the output HIV-1 NDK virus produced by BDM infected with 10 to 1000 ng of $p24^{gag}$ reached about 0.5% of the amount of input virus (Table 1). Taken together, HIV-1 NDK and HIV-1 LAV were about 10^4 and 10^5 times more infectious, respectively, for T lymphocytes than for macrophages.

To understand the mechanism responsible for the different outcomes of infection of BDM with HIV-1 LAV and HIV-1 NDK, we tried to determine at which step of the virus cell cycle the differences occurred and which gene(s) is responsible for its control. For this purpose, BDM were infected with several recombinants from our collection of chimeric viruses (Fig. 1) and replication was

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TABLE 1

Replication of HIV-1 in PBL and BDM Cells[a]

HIV-1	PBL p24^{gag} (ng/ml)	BDM p24^{gag} (ng/ml)	BDM $TCID_{MT4}$/ml
Input virus[b]			
PAR	10.0	0.01	ND
NDK	0.001	10.0	10^4
LAV	0.01	100.0	10^5
Output virus[c]			
PAR	28	89.5	<10
NDK	358	3.6	10^3
LAV	391	0.29	$\leqslant 10$

[a] PBL (10^6) or BDM (5×10^5) cells were infected in a final volume of 1 ml as described previously (*7*).

[b] Minimal infectious dose of HIV-1 necessary for productive infection of 10^6 PBL or 5×10^5 BDM cells were determined by end-point dilution of viral stock. Infection with a higher dilution of input virus did not result in virus production. p24^{gag} was determined by the antigen capture technique (Coulter, Inc., Hialeah, FL; HIV-1 p24 antigen assay), $TCID_{MT4}$ by titration in MT4 cells (*26*). HIV-1 LAV and NDK were prepared in PBL. Macrophage-tropic strain HIV-1 PAR (*29*) was prepared in cord blood lymphocytes (*7*).

[c] Output virus production was determined at the peak of virus production after infection of 10^6 PBL or 5×10^5 BDM cells with 600 ng of p24^{gag} of each input virus.

monitored by PCR (Fig. 2). High sensitivity of HIV-1 replication to AZT, a known inhibitor of reverse transcriptase, demonstrated that a PCR signal reflected newly synthetized HIV-1 DNA and not HIV-1 DNA which originated from virus stocks either in a free form or present within infectious virions (Fig. 2). All recombinants bearing the HIV-1 NDK envelope glycoproteins (Fig. 1, lines b, d, e, h, i, j, and k) entered BDM about 10 times more efficiently (Fig. 2) than recombinants with envelope glycoprotein of HIV-1 LAV (Fig. 1, lines a and c). The amplification signal from BDM infected with HIV-1 NDK was about 10 times weaker than that from BDM infected with control macrophage-tropic strain HIV-1 PAR. This result suggests that *env* gene variants occurring among different T-cell-tropic strains have an importance for virus entry into BDM that is similar to that of those occurring between T-cell-tropic and macrophage-tropic viruses.

Ample experimental evidence (*25–28*) has shown that HIV-1 NDK has more pronounced syncytia-inducing (SI) phenotype than HIV-1 LAV. Such a phenotype of HIV-1 NDK could also be predicted from comparison of the amino acid sequences of V2 and V3 regions of gp120 of both viruses (*4, 5*). Therefore, according to the classification of SI/NSI isolates, HIV-1 LAV could be expected to be more macrophage-tropic than HIV-1 NDK. This contrasts with experimental results and suggests that another structural feature(s) of the HIV-1 NDK envelope glycopro-

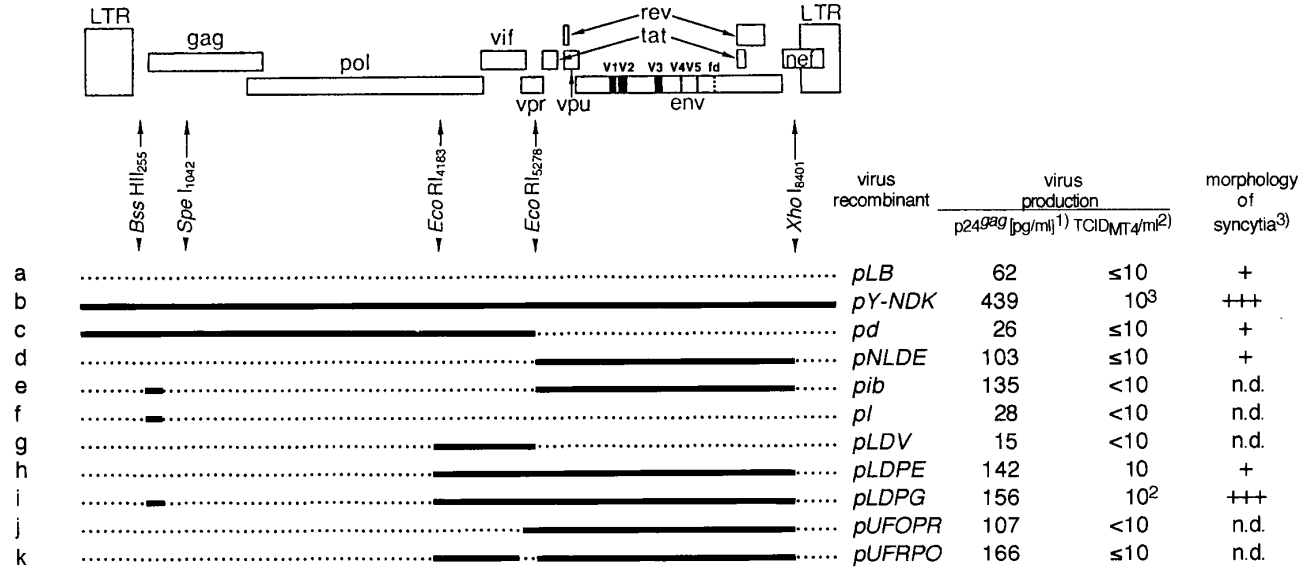

FIG. 1. Genetic control of BDM infection with T-cell-tropic viruses. Recombinant provirus molecules derived from DNA cloned from HIV-1 LAV (dotted lines) and HIV-1 NDK (solid lines) were constructed by reciprocal exchange of genetic material using conserved *Bss*HII$_{255}$, *Spe*I$_{1042}$, *Eco*RI$_{4183}$, *Eco*RI$_{5278}$, and *Xho*I$_{8401}$ sites (*25–27*) or by PCR-directed recombination (*23*). Recombinants *UFOPR* (line j) and *UFRPO* (line k) constructed by PCR-directed recombination were obtained from recombinant *LDPE* by reciprocal exchange of HIV-1 LAV and NDK fragments *Eco*RI$_{4183}$–5095 and 5153–*Eco*RI$_{5278}$, respectively. They contained complete *vpr* (*UFOPR*) and *vif* (*UFRPO*) genes of HIV-1 NDK. The positions of restriction sites are indicated for the HIV-1 NDK sequence. Chimeric viruses were obtained by cocultivation of transfected COS cells with PBL (*25*). Columns under virus production: (1) Virus production from BDM infected with 100 ng of p24^{gag} of input virus; (2) Virus titer, expressed as $TCID_{MT4}$, was determined in an end-point dilution assay of BDM-free supernatants obtained 15 days p.i. as measured by formation of cytopathic effects (CPE) in MT4 cells. $\leqslant$, CPE was found only in nondiluted supernatants of some experiments. (3) The fusogenicity of different recombinant viruses was determined by syncytia formation on MT4 cells (*25*). Subcultures of MT4 cells infected with different recombinants were coded and the sizes of syncytia were scored independently by two researchers each 3rd day over a 2-week period. The morphology of syncytia is indicated by + for standard-size syncytia induced by the HIV-1 LAV prototype and by +++ for large syncytia induced by the highly cytopathic strain HIV-1 NDK. n.d., not determined.

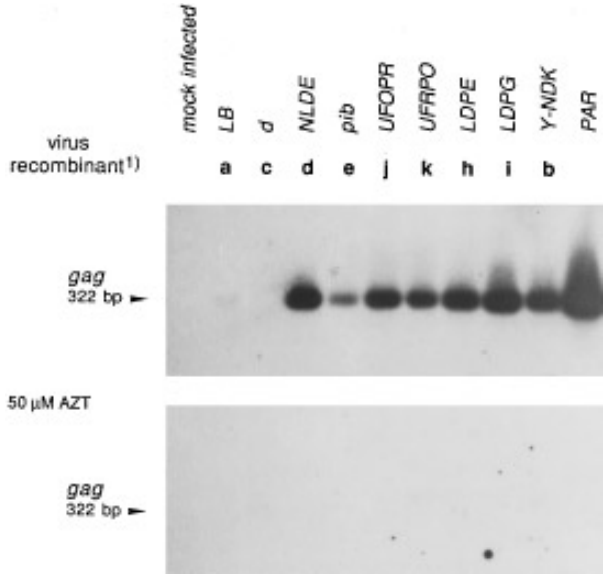

FIG. 2. HIV-1 DNA synthesis in BDM infected with lymphotropic recombinant viruses. BDM (5×10^5) were infected with about 100 ng of $p24^{gag}$ of recombinant HIV-1 (Fig. 1) or macrophage-tropic virus HIV-1 PAR. All viral stocks were treated with 200 U of DNaseI/ml for 30 min at room temperature before use. Retrotranscription of HIV-1 genomic RNA was monitored by DNA amplification 48 hr p.i. Cell lysates were prepared and the amplification reaction was carried out as described previously (*7*). Intermediate *gag* primers (*30*) were localized at the following positions of HIV-1 LAV sequence: *gag* + primer A1 (882–909), *gag* − primer A2 (1177–1204), and *gag* probe CBIO (949–976). PCR amplification products of *gag* gene were visualized after Southern blot hybridization with ^{32}P-labeled oligonucleotide probes. Amounts of genomic DNA present in different cell lysates were detected by PCR of the first intron of the human β-globin gene (*28*). The quantity of PCR products was estimated according to decadic dilutions of Gene Amplimer HIV-1 Positive Control DNA (Perkin–Elmer Cetus, Inc., Norwalk, CT).

tein(s) is involved in interaction with BDM. We have shown previously (*28*) that the envelope glycoprotein is involved in the enhanced resistance of HIV-1 NDK to treatment of target cells with OKT4-A anti-CD4 MAb and suggested that HIV-1 NDK might be able to use a CD4-independent mechanism of virus/cell entry into CD4-positive cells. Such a mechanism could be responsible for the efficient entry of HIV-1 NDK in BDM.

To characterize the region(s) of the HIV-1 genome responsible for the control of productive infection of BDM with T-cell-tropic virus, we infected BDM with viral recombinants and followed their replication kinetics by $p24^{gag}$ antigen capture assay (Figs. 1 and 3). In seven independent experiments in which macrophages from two healthy donors were used, the highest quantity of capsid antigen was produced by parental clone HIV-1 Y-NDK. It ranged from 0.2 to 1% of the input virus and was stable for about 4 weeks p.i. Levels of $p24^{gag}$ antigen detected in supernatants of BDM infected with macrophage-tropic virus HIV-1 PAR were about 50 times higher. Virus production was highly sensitive to the presence of AZT in the culture medium (Fig. 3). Small amounts of $p24^{gag}$ were transiently detected during the first 5 days of the cultivation period in the presence of AZT. They ranged from 10 to 30% of the control value, probably due to a release of the input virus which was adsorbed but failed to be internalized by BDM. $p24^{gag}$ amounts were reduced to less than 2% after the first change of AZT-containing medium. This means that the great majority of $p24^{gag}$ detected after infection of BDM with T-cell-tropic viruses corresponded to newly synthesized virus.

Recombinants *LDPE* and *LDGP* (Fig. 1, lines h and i), which contained the HIV-1 NDK region $p34^{int}$-*env* (fragment *Eco*RI$_{4183}$–*Xho*I$_{8401}$) reproducibly produced more $p24^{gag}$ antigen than the recombinant *NLDE* (Fig. 1, line d), bearing a shorter HIV-1 NDK fragment *Eco*RI$_{5276}$–*Xho*I$_{8401}$. All recombinants which contained the HIV-1 LAV envelope glycoprotein produced at least 10 times less of the virus (Fig. 1, lines a, c, f, and g and Fig. 3) in agreement with the lower efficiency of their entry into BDM. These results confirm that (i) envelope glycoprotein(s) of T-cell-tropic viruses genetically controls restriction of productive infection at the virus/cell entry, and suggest that (ii) additional genes different from *env* are necessary for an efficient replication of HIV-1 NDK after its entry in BDM.

In seven independent experiments, BDM infected with 100 ng of the HIV-1 NDK $p24^{gag}$ produced 10^2 to 10^3 tissue culture infectious units (Figs. 1 and 4) as determined in the MT4 assay ($TCID_{MT4}$). In BDM infected with HIV-1 LAV, infectious progeny was only detected in nondiluted supernatants in three of seven experiments. Phenotypic analysis revealed that reciprocal exchange between HIV-1 LAV and NDK *env* gene (*Eco*RI$_{5278}$–*Xho*I$_{8401}$ fragments) resulted in recombinants that were not able to form a higher quantity of infectious progeny than the prototype virus (Fig. 1, lines c and d). This indicates that parts of the HIV-1 NDK genome inside as well as outside of this fragment have contributed to the production of an infec-

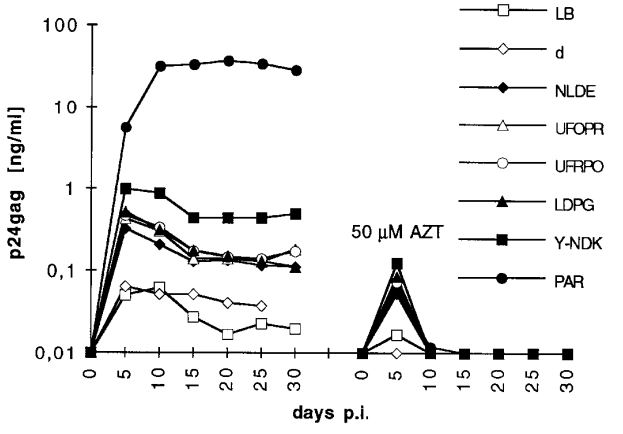

FIG. 3. Replication kinetics of recombinants of T-cell-tropic HIV-1 in BDM. BDM (5×10^5) were infected with about 100 ng of $p24^{gag}$ of recombinant HIV-1 (Fig. 1) or macrophage-tropic virus HIV-1 PAR. Virus production was determined by $p24^{gag}$ antigen capture assay. The cutoff value was 7 pg/ml. The culture medium was completely changed every 5 days.

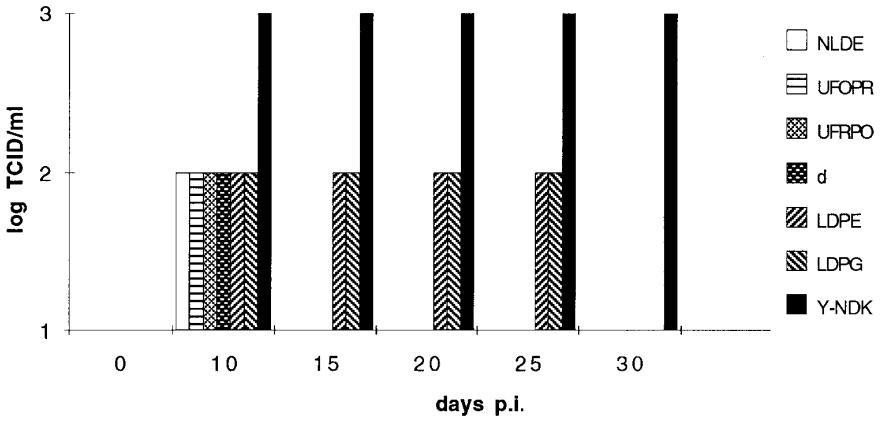

FIG. 4. Production of an infectious progeny from BDM infected with T-cell-tropic recombinant viruses. BDM (5×10^5) were infected with about 100 ng of $p24^{gag}$ of recombinant HIV-1 (Fig. 1) and the infectious titer was determined by MT4 assay. The culture medium was completely changed every 5 days. The cutoff value in these experiments was equal to 10 $TCID_{MT4}$/ml.

tious progeny observed in the case of HIV-1 NDK. Also, BDM supernatants infected with recombinants containing the HIV-1 NDK *MA* gene, individually or simultaneously with the HIV-1 NDK *Eco*RI_{5278}–*Xho*I_{8401} fragment (Fig. 1, lines e and f) or the HIV-1 NDK fragment *Eco*RI_{4183}–*Eco*RI_{5278} (Fig. 1, line g), remained noninfectious. Only recombinants containing the HIV-1 NDK region $p34^{int}$-*env* (fragment *Eco*RI_{4183}–*Xho*I_{8401}, Fig. 1, lines h and i) produced virus infectious for T lymphocytes in BDM cell-free supernatants. Selective advantage of HIV-1 NDK in CD4-positive lymphocytes is related to variations in *gag–pol* genes (*26*) and in the *MA* gene together with the gene coding for the envelope glycoprotein (*23, 28*). Very different results obtained in BDM provide an intrinsic control which argues against the possibility that contaminating lymphocytes in the macrophage cultures contributed to the observed patterns of replication of T-cell-tropic viruses in BDM.

The genetic structure of the fragment *Eco*RI_{4183}–*Eco*RI_{5278} is quite complex. In addition to the *vif* gene, it contains the N-terminal portion of the *vpr* gene and the C-terminal portion of $p34^{int}$ gene. In order to characterize more precisely the genetic region(s) of HIV-1 NDK responsible for infection of BDM, genetically defined *vif* and *vpr* recombinants were constructed by PCR-directed recombination within *Eco*RI_{4183}–*Eco*RI_{5278} fragment (Fig. 1, lines j and k). *vif* and *vpr* genes present in the prototype virus HIV-1 LAV (parental clone *LB*) are isogenic with the genes present in the macrophage-tropic recombinant virus NF462 (*14*). Therefore, both genes are fully compatible with replication of HIV-1 in BDM. Reciprocal exchange of the HIV-1 LAV and NDK genes *vif* and *vpr* in the provirus *LDPE* produced the recombinants *UFOPR* and *UFRPO.* Both recombinants produced intermediate quantities of $p24^{gag}$ between HIV-1 *NLDE* and *LDPG* (Fig. 3). More significant differences were found in the production of infectious progeny from BDM (Fig. 4); the transient production of the HIV-1 *UFOPR* and *UFRPO* infectious progeny ceased 15 days before that of HIV-1 *LDPG* and *LDPE.* Among the 192 amino acids (aa) of Vif, 15 differ between HIV-1 LAV and HIV-1 NDK. Some of these substitutions, localized at both terminal portions of Vif (R → S, aa 23; K → N, aa 36; D → R, aa 37; K → A, aa 155; Q → K, aa 158; H → R, aa 183), could be important for protein function. In contrast, only one amino acid substitution (N → S, aa 41) is present in the N-terminal portion of Vpr. Despite the high variability in the *vif* gene and low variability in the *vpr* gene, both HIV-1 NDK genes contributed, in addition to the *env* gene, to the enhanced capacity of HIV-1 NDK to produce infectious progeny in BDM.

Our results indicate that two genetic mechanisms, one effective at virus entry and the other after entry, determine differences in replication of T-cell-tropic viruses in BDM. These mechanisms are controlled by the envelope glycoprotein gene and by the accessory genes *vif* and *vpr.* Both accessory genes *vif* and *vpr* are involved in early steps of virus cycle before formation of HIV-1 provirus (*12, 18*). In comparison with the majority of previous studies aimed at investigating restriction either at the virus entry level or after entry, we have been able to demonstrate and quantitate both restriction mechanisms in one homogeneous system of recombinants. A principal role of the *env* gene in the control of the tropism for macrophages and T cells at the virus entry level has been described by many groups of investigators (*1–6*). The mechanism and genetic control of restriction after entry is much less well documented. The present results indicate that a higher functional activity of *vif* and *vpr* alleles of HIV-1 NDK is related to its ability to more easily overcome postentry restriction in BDM. Role of accessory genes in the control of macrophage-tropic phenotype has previously been studied by inactivation of gene function by *in vitro* mutagenesis (*13–16*). In the present report we have compared activities of "wild-type" alleles of *vif* and *vpr* genes of HIV-1 LAV and NDK. Our results provide evidence that natural variation in the wild-type alleles of *vif* and *vpr* genes is related to quantitative differences in phenotypic behavior of HIV-1 strains. Both types of

restriction, that effective at the virus entry level and that effective after entry, could influence HIV-1 tropism to different extents.

HIV-1 NDK is able to productively infect a remarkably wide spectrum of host cells and shows a highly flexible replication strategy. Different genes and their combinations confer on HIV-1 NDK a selective advantage over the prototype virus in different cell systems. Variations in *gag–pol* genes are related to the rapid replication in CD4-positive lymphocytes (*26*) and to the productive infection of intestinal cells HT29 (*24*). The N-terminal 75 amino acids of the HIV-1 NDK matrix protein $p18^{gag}$ together with the gene coding for HIV-1 NDK envelope glycoproteins are responsible for the enhanced fusogenicity of HIV-1 NDK in CD4-positive lymphocytes as well as the enhanced infectivity of HIV-1 NDK in CD4-negative cell lines HACAT and RD (*23, 28*). On the other hand, productive infection of BDM depended on variations in the *vif* and *vpr* genes. In a linguistic metaphor, the HIV-1 NDK model clearly shows that identical text of genetic information can be interpreted in different ways according to the context of host cells.

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